Miller (W. D.)

FERMENTATION

IN THE

HUMAN MOUTH

ITS RELATION TO CARIES OF THE TEETH.

THE INFLUENCE OF ANTISEPTICS, FILLING MATERIALS, ETC., UPON THE FUNGI OF DENTAL CARIES.

THE FUNGI OF DENTAL CARIES; THEIR PURE CULTIVATION AND EFFECT UPON LOWER ANIMALS.

By Dr. W. D. MILLER, Berlin, Germany.

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FERMENTATION IN THE HUMAN MOUTH; ITS RELATION TO CARIES OF THE TEETH.

BY DR. W. D. MILLER, BERLIN, GERMANY.

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During the last two years I have stated at different times and places, as the result of many experiments, that "the first stage of dental caries consists in a decalcification of the tissue of the teeth by acids, which are for the greater part generated in the mouth by fermentation." The object of the investigations described in this and the following papers is to determine this ferment, and the conditions essential to its action. I shall seek in what follows to present no views which are not the legitimate and necessary results of rigid and exact experiment, and I shall give in detail a description of each series of experiments, in order that every one may have an opportunity to judge of the accuracy of the work and the justice of the conclusions drawn from it.

It is, nevertheless, with some hesitancy that I venture to present before the dental profession the results of my last six months' labor, having learned by experience the almost endless number of agents which combine to vitiate such a series of experiments as that which I am about to offer, and the exceeding great care which is necessary in excluding or eliminating all irrelevant factors.

If, therefore, I have been guilty of any oversight, or failed to take all possible precautions to guard against error, I hope that some one will kindly show me where I have gone astray, and put me in the right course again.

The larger apparatus necessary for these experiments are :

1. A large double-walled incubator, with gas regulator for maintaining any desired constant temperature.

2. A Koch sterilizer.

3. A damp chamber. (See Fig. 1.)

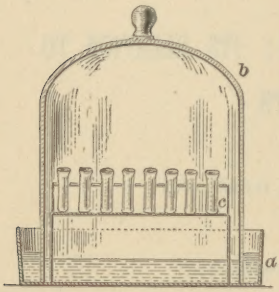


FIG. 1.—Damp Chamber. *a*, shallow glass vessel partially filled with water. *b*, glass globe lined with wet bibulous paper. *c*, metallic stand for culture tubes.

4. A drying oven for sterilizing instruments, glass vessels, etc., at a temperature of one hundred and fifty degrees Centigrade.

5. A good microscope, with either water or oil immersion.

It is not necessary to mention the smaller instruments, glass vessels, etc., etc., nor the apparatus necessary for making a chemical analysis of the products of the fermentation ; these are sufficiently familiar to every one.

To avoid repetition, I will say here that *all* vessels and instruments used in the culture experiments were purified in the flame of a Bunsen burner, when practicable, otherwise by exposing for fifteen minutes in the drying oven to a temperature of one hundred and fifty to one hundred and sixty degrees Centigrade, (three hundred and two to three hundred and twenty degrees Fahrenheit), and that all substances used as culture substrata were sterilized four times by exposure, at intervals of twelve hours, for half an hour, to steam at one hundred degrees Centigrade, in a Koch sterilizer. Furthermore, all infections from carious dentine were made as follows : The cavity of a freshly extracted carious tooth is cleared of food, and carefully brushed over with a pledget of cotton dipped in carbolic acid (ninety per cent). The acid is then thoroughly absorbed by means of bibulous paper, and layer after layer of the soft dentine removed with a repeatedly purified instrument, until the deeper parts are reached ; then, a portion of the clean soft dentine, scarcely as large as a pin-head, is removed, and quickly brought into or upon the culture medium.

Infections from the mouth were made by scratching upon the surface of the mucous membrane of the cheek, or the margin of the gum, with the end of a clean platinum wire, and then dipping it into the culture medium. The materials used for culture were :

No. 1. Sterilized saliva 50,0
Sugar 1,0
Starch 0,5

No. 2. Sterilized milk.

No. 3. Decoction of malt 50,0
Sugar 1,0

The malt decoction is made by boiling, with slight evaporation, 20,0 dry malt with 120,0 water for ten minutes, and filtering.

No. 4. Sterilized saliva 50,0
Water 50,0
Starch 20,0
Sugar 2,0

The starch is added to the cold solution of water and saliva, and stirred until it becomes evenly divided throughout the solution ; it is then poured into shallow glass vessels with glass covers, and put into the sterilizer for complete sterilization ; it there congeals and forms a solid mass, upon the surface of which the infections may be made. It possesses all the advantages of gelatine, with one great additional one, in that it does not liquify at blood temperature.

No. 5. Decoction of malt 100,0
Sugar 2,0
Starch 20,0

Prepared in the same way as No. 4.

No. 6. Beef extract 2,0
Water 100,0

No. 7. Water 100,0
Beef extract 2,0
Sugar 2,0

No. 8. Fresh baked potato, cut into slices one half inch thick, with a clean knife.

Other substances were used, but need not be considered here. Additional sugar is not absolutely necessary where malt is used, though I have, so far, obtained better results by adding a small quantity. The kind of sugar is immaterial, provided it be fermentable; even cane sugar, though not directly fermentable, is converted into a fermentable variety in the culture. Where small quantities of any culture material were used, the cultures were kept in the damp chamber to prevent their drying up or becoming too concentrated by evaporation. All cultures were made under a temperature of thirty-six to thirty-eight degrees Centigrade.

We will begin with the fundamental experiments.

Exp. 1. Fresh saliva is mixed with sugar or starch, one to forty, and kept at blood temperature. It invariably becomes acid in four to five hours. But some one, no doubt, will say that this is a result of no consequence, because the experiment was not made within the oral cavity; for his personal benefit we give the following:

Exp. 2. A glass tube two c. m. long and three m. m. wide, is filled with starch, sterilized, and fastened to a molar tooth in the mouth on going to bed; next morning the contents of the tube will have a strong acid reaction. A cavity in a tooth, or a piece of linen, which may be saturated with a solution of starch, will answer the purpose as well as the glass tube. That the acid is the same in each case will be further established below.

Exp. 3.—The mixture of saliva with starch or sugar, is kept for a half-hour in the sterilizer at one hundred degrees Centigrade, and then placed in the incubator; it does not become sour in four, nor in twenty-four hours; in fact, not at all. We conclude that the ferment is rendered inactive by a temperature of one hundred degrees Centigrade.

Exp. 4. The starch is heated to one hundred and fifty degrees Centigrade before mixing with the saliva; the solution still becomes sour. Conclusion: the ferment exists, not in the starch, but in the saliva.

We have now to determine the question: Is it an organized ferment (fungi), or is it an unorganized ferment (ptyaline)?

This question is determined by the following experiments:

Exp. 5. From six to eight grams of saliva are agitated in a test-tube with as much sulphuric ether as it will take up, starch added, and the whole put in the incubator. On examination after a few hours, we will find sugar in the solution, but no acid; in other words, the acid-forming ferment has been rendered inactive, but the unorganized, sugar-forming ferment, not.

Exp. 6. Instead of ether, enough carbolic acid is added to make the solution one-half per cent. strong; the result is the same. These two experiments show that the ptyaline of the saliva (which was not injured by the presence of the ether or the carbolic acid, as proved by the fact that it retained its diastatic action) is not the cause of the acid reaction.

Exp. 7. According to Paschutin, ptyaline is devitalized by exposure twenty minutes to a temperature of sixty-seven degrees Centigrade. Organized ferments could not be killed by the same means. We accordingly subject a mixture of saliva and grape-sugar to the given temperature for twenty minutes. We thereby destroy the ptyaline; the mixture, nevertheless becomes sour if allowed to stand in the incubator for twenty hours.

This experiment confirms the result of experiments five and six, and we begin to suspect that we have to deal with an organized ferment. This supposition is confirmed by the following experiment.

Exp. 8. Six to eight drops of a perfectly sterilized solution of sugar in saliva (1-40), in a miniature test tube with cotton cork, are infected from the mouth, or with carious dentine, as described above; in twenty-four hours the solution will be acid; with a fraction of a drop of this solution a second tube is infected; it will likewise become acid; from this a third, etc., etc.; each becomes acid in turn, while the control tube (containing the same solution, not infected) remains neutral.

The conclusion is plain, that we have to do with a ferment which is capable of reproducing itself; in other words, an organized ferment. It therefore becomes evident that not only free in the mouth, but in the deeper parts of carious dentine, we have a

fungus which is capable of producing an acid reaction in characteristic substrata.

Exp. 9. Each of thirty small tubes were furnished with eight drops of solution No. 1, and each of thirty other tubes with as many drops of solution No. 3, and all were sterilized. Twenty-four were then infected from the mouth, twenty-four with carious dentine, and twelve were left as controls.

In twenty-four hours all forty-eight of the infected solutions were acid, while the twelve controls remained neutral.

Exp. 10. Make a solution of 40,0 of saliva and 1,0 of starch; put equal portions in two flasks, *a* and *b*, and cover the surface of the solution in *a* with a layer of pure oil to prevent the free access of air, or:

Exp. 11. Place flask *a* in an air-tight bottle containing a fresh alkaline solution of pyrogallic acid (which abstracts the oxygen from the air), or:

Exp. 12. Exhaust flask *a* by means of the air pump, so as to produce a tolerably complete vacuum. The quantity of acid produced in *a* will be, on an average, the same as that produced in *b*.

We conclude from experiments eight, nine, and ten, that the fungus in question is independent of the free access of air or oxygen for its development and characteristic action, a conclusion which would exclude the fungus of vinegar, (*mycoderma aceti*) and which is of the utmost practical importance, since it signifies that this fungus can develop and perform its work deep in the dentinal tubules, or under fillings, provided the necessary materials are furnished it.

Exp. 13. Place a piece of carious dentine upon the surface of the culture material described in number four, five, or six; in twelve hours the dentine will be surrounded by a white ring, from four to eight m. m. in diameter; the material within this ring will be partially liquified, and have an acid reaction. The same result follows when the infection is made from the mouth.

Exp. 14. Produce 10,0 of saliva by chewing a sterilized quill toothpick, add 0,5 starch or sugar, and place in the incubator. Then give the oral cavity a most thorough cleansing with pure

water, using toothpick, brush and floss, the object being to free the mouth from micro-organisms as completely as possible. Then produce again 10,0 saliva, add 0,5 starch or sugar, and put in the incubator. The amount of acid produced in a given time will, in the latter case, be often as low as one-fourth of that in the former. Conclusion: By thoroughly cleansing the mouth we no doubt remove the greater portion of the fungi, hence the small amount of acid produced. By using strong antiseptics, or by repeatedly filtering the saliva, we may reduce the amount of acid produced in twenty-four hours almost to 0. An experiment yet to be made is to take the saliva direct from the gland, before it becomes infected with the organisms of the mouth; it should not then become sour when mixed with starch and allowed to stand at blood temperature. In every case a careful microscopic examination of the cultures was made, revealing the constant presence of a fungus, chiefly in the form of diplococci, either single or in chains, less often in form of bacteria, bacilli, or even threads. (See Fig. 2.)

Sometimes all these forms are found on a single thread, thus proving what I have already demonstrated for *Leptothrix buccalis* and *Leptothrix gigantea* (Miller), the genetic connection of these different forms. The particular form in which the fungus occurs depends somewhat upon the culture medium, as well as upon the age of the culture. By using a glass tube as culture vessel we may demonstrate that whether the culture is made in the mouth or out of it, under similar conditions the fungus is the same. The fungus is not capable of producing an acid reaction of all substances in which it may vegetate. A luxuriant growth may be obtained in beef extract, but no acid is produced, unless sugar is present.

FIG. 2.—Some of the forms in which the fungus, treated of in this article, occurs.

It is only from carbo-hydrates (especially sugar) that it appears to be able to produce acid in any considerable quantity, or at all. This question, however, as well as the morphology, physiology, development and life-conditions of the fungus, will receive consideration in a separate number.

We have, then, a micro-organism which agrees morphologically with the *Bacterium acidi lactici*, and which, without the presence of oxygen, produces acid from sugar, so that we would probably not be far from right if we were to say that the organism in question is simply the fungus of lactic acid; we will, however, reserve our decision for the following number, where the analysis of the product of the fermentation will be given, that being the one *sure* method for determining the species of any ferment bacterium.

In all cultures, it is of course essential that the culture-substratum be neutral when the inoculation is made; should it be acid it must be neutralized. This is best accomplished by very carefully adding the carbonate of sodium. Without this precaution it would be somewhat difficult to determine whether acid had been produced by the action of the fungus or not.

In the light of these experiments the thorough decalcification of the tooth substance in caries is easily accounted for. The saliva is, no doubt, always, particularly in mouths of uncleanly persons, impregnated with sugar, either taken directly into the mouth, or formed there by the action of the ptyaline of the saliva upon starch. The question of the presumable diastatic action, as well as of a presumable inverting power on the part of the organisms themselves, will be considered in the chapter on Physiology.

Wherever this stagnates between the teeth, in fissures, etc., etc., especially during sleep, it *must* become acid. When a portion of the dentine has become decalcified, it, as is well known, takes up the liquids of the mouth and the fungi with them like a sponge, and the fungi, being independent of the free access of air, go on producing acid within the dentinal tubules. As each layer of dentine becomes softened in turn, the micro-organisms *follow after*, continually producing new acid. Hereby the zone of softened, non-infected dentine is readily understood. The production of acid is

entirely independent of the reaction of the saliva as it enters the mouth, hence the uselessness of "testing the saliva" for acid. That the liquid squeezed out of the tubules of *decaying* dentine has an acid reaction, every dentist in America who has a piece of blue litmus paper and is not color blind can easily prove for himself.

The result of experiment six plainly shows one cause of the good effects which the profession has seen from the use of carbolic acid.

The fact that a pure culture was obtained in most cases by the first inoculation seems to indicate that the fungus exists in a state of tolerable purity in the deeper parts of the carious dentine. This question will, however, receive consideration later. The action of the fungus upon substances which contain no carbo-hydrates will also be considered under Physiology.

In addition to these experiments, I add the following: A sound bicuspid tooth was sawed into sections, varying from one-third to one m. m. in thickness, and an equal number of these sections placed in each of two test-tubes. Into one of these test-tubes were then brought five c. c. of a perfectly neutralized two per cent. aqueous solution of beef extract; into the other the same solution, with the addition of 0,2 cane sugar. Both tubes with their contents were then sterilized, and, upon cooling, infected from a pure culture of the fungus under consideration.

The solution in the second tube became acid in a few hours; not so, however, with that in the first tube, it being non-fermentable. At the end of one week the thinner sections in the second tube were so far softened that one of them, removed for examination, could be easily bent between the fingers. At the end of the second week all but the thicker sections were completely decalcified. One of these sections was now placed upon the freezing microtome and made into cuts, which were stained in fuchsine and mounted in Canada balsam. A microscopic examination showed that the fungi had penetrated many of the tubules to a considerable depth, the invaded tubules being at the same time slightly extended. At the close of the third week the invasion was found to have become much more extensive, the tubules much dilated, and in some places

the walls were broken through, leading to the formation of oval spaces or caverns in the dentine.

In short, we had a typical case of caries.

It is hardly necessary to state that the thinnest sections in the first tube, where the development of the fungus was not accompanied by an acid fermentation, did not show even the traces of softening, to say nothing of caries.

I had then produced caries by inoculating sound dentine from a pure culture of a fungus found in carious dentine, in the presence of the same fermentable substances that occur in the mouth. It seems that a clearer solution of the problem can at present scarcely be expected. Of course the thought at once suggests itself to every one that this decay is quite independent of putrefaction; all evidence points to the conclusion that putrefaction at most does nothing more than dispose of the already devitalized and much riddled remains of tissue, and we are in danger of overrating its influence, even at this stage.

Pieces of dentine in a solution kept constantly pure and sour by fermentation, not only become softened and show the microscopic changes characteristic of carious dentine, but finally, after some months, disappear altogether, as has repeatedly been the case in my cultures. From this we must infer that the process commonly known as putrefaction is absolutely essential at no stage of caries; especially is this the case in caries of enamel.

It has been intimated that the active agent in this process is nearly related to, if not identical with, the fungus of sour milk: *Bacterium acidi lactici*. The analysis of the product of fermentation will show the truth or falsity of this supposition.

The method of carrying out such an analysis will now be given. Two hundred c. c. fresh saliva are mixed with 2,0 starch and allowed to stand forty-eight hours at blood temperature; the mixture is then filtered and heated to one hundred degrees Centigrade, to stop the fermentation. This process is repeated until about a litre of the solution has accumulated. It is then placed in a retort and reduced to a volume of about seventy-five c. c. It will be very strongly acid. A few drops of this liquid are added to a thin solution of methyl-violet, and leave it unchanged; from this we

conclude that we have to deal with an organic acid, as an inorganic acid would turn it first blue, and then green. Since the acid did not distill during the prolonged boiling, we may set it down as non-volatile; hence a non-volatile, organic acid. The distillate was very slightly acid; we will call it distillate number one, as we wish to refer to it again.

The solution was further reduced in volume to about forty c. c. over the water-bath, and then transferred to a large glass vessel, briskly shaken with one and one-half to two litres of sulphuric ether, and allowed to stand until the ether became perfectly transparent. This was then filtered into a large retort and distilled, proper precautions being observed to prevent accidents. When the volume had been reduced to about fifty c. c. the solution was filtered into a porcelain vessel, and still further reduced over the water-bath. A portion of the solution tested in the short tube of a Mitscherlich double-shadow polaristrobometer gave, as a mean of nine readings, a rotation of the plane of polarization equal to 0,015 degrees, or 0° 0′,9. In other words, the solution was optically inactive, the 0° 0′,9 being far within the range of the error of experiment, especially as the solution was not absolutely transparent.

An excess of freshly prepared oxide of zinc was then added to the solution, and the whole slowly and carefully boiled, water being added as it was found necessary, till the reaction became neutral, or nearly so, filtered into a large glass evaporating dish, and put away at the temperature of the room for the salt to crystallize. A drop of this solution placed upon a glass slide gave, upon crystallization, the forms seen in figure one, which are at once recognized as crystals of lactate of zinc. In a few days a quantity of a whitish crystalline powder had formed. This was placed upon a filter, the mother-liquid squeezed out, washed in absolute alcohol, dissolved in hot water, re-crystallized and dried over sulphuric acid; it then weighed 0,343. After exposing to a temperature of one hundred degrees Centigrade, or a little more, till the weight became con-

Fig. 1

stant, it weighed 0,2816; it lost accordingly 17,9 per cent.* of water of crystallization, corresponding to three molecules of water. The salt was then dissolved in water, the zinc precipitated as carbonate and burned. The burned mass (zinc oxide) weighed 0,0970. We have consequently:

Substance analyzed (a zinc salt) = 0,343
Oxide of zinc = 0,097

The zinc oxide is seen to be equivalent to 28,2 per cent. of the substance analyzed.

The formula for the inactive ethylidene lactate of zinc is—

$$\left.\begin{matrix} C_3 H_5 O_3 \\ C_3 H_5 O_3 \end{matrix}\right\} Zn + 3 H_2 O, = 243 + 54.$$

Dried at ordinary temperature it contains 27,3 per cent. zinc oxide. The result obtained from the analysis differs, therefore, from that deduced from the formula by less than one per cent., and settles beyond doubt the fact that the substance analyzed was the lactate of zinc, or that the acid generated by the fermentation is lactic acid, or, more exactly, inactive ethylidene lactic acid, since, as shown above, the acid solution was optically inactive, and the zinc salt contained three molecules of water of crystallization. The salt was furthermore soluble in sixty-two parts water at fourteen degrees Centigrade.

I repeated the analysis with the following solution:

Water, 1000 c. c.
Saliva, 300 c. c.
Bouillon, 200 c. c., made by boiling 125,0 beef ten minutes in 300 c. c. of water.
Sugar, 10,0.

This solution being slightly acid was neutralized with the carbonates of lime and sodium, sterilized, and infected from a pure culture of the fungus in question.

It was treated throughout exactly in the manner above described, except that the zinc salt was converted into the sulphide instead of the carbonate, and burned with powdered sulphur in a stream of hydrogen. The result was as follows:

* Theoretically 18,2, or 0,3 per cent. more.

Substance analyzed, = 1,0540
Zinc sulphide, = 0,415
Zinc, = 26,38 per cent.
instead of 26,74 per cent., as deduced from the formula, a difference of only one-third of one per cent.

In this case the substance was dried at one hundred degrees Centigrade before weighing, and the formula becomes

$$\left.\begin{matrix} C_3\,H_5\,O_3 \\ C_3\,H_5\,O_3 \end{matrix}\right\} Zn = 243.$$

One more analysis was made, using—

Water, 1000 c. c.
Liquid beef extract, 20 c. c.
Sugar, 10,0.

The result was the same, and need not be given; the two analyses above described being abundantly sufficient to show that the acid generated by the fungus in question is the common ferment, lactic acid.

Distillate number one, referred to above, owed its slight acidity, we now know, in part at least to lactic acid, since, when an aqueous solution of lactic acid is boiled, a small fraction of the acid goes over with the water. To ascertain, however, whether any other acid, especially volatile, was present, the distillate was boiled with carbonate of lime, filtered, evaporated to dryness, a small amount of dilute sulphuric acid added, and heated in a retort over the water-bath; a few drops of an oily acid came over, which, when taken upon the fingers, smelled like butyric acid; the amount, however, was so small, that no attempt could be made to analyze it.

I have been able with some degree of certainty to establish the presence of lactic acid in carious dentine, by a method theoretically so simple that it seems strange it has never been made use of before, but which, however, in practice is only carried out with great difficulty. My first and second attempts were only partially successful; the third succeeded sufficiently well to justify its description here.

In this experiment I made use of fifteen teeth, all containing considerable quantities of carious dentine, and all extracted on the day of use. The remains of food were first removed from the

cavities, but none of the softened dentine; then all the softened dentine was taken out and placed in a porcelain vessel, cut or picked into fine pieces, placed in a test-tube with one c. c. of water, and two drops of a ten per cent. solution of hydrochloric acid added. Any free lactic acid in the carious dentine would remain free, and any existing in combination with lime would be set free by the hydrochloric acid. It was then gently shaken with about twenty-five c. c. sulphuric ether, and the latter, holding the lactic acid in solution was, after some minutes, poured off into a second test-tube; here it must be allowed to stand from twenty-four to forty-eight hours, till it becomes perfectly clear. It was then filtered into a porcelain dish, evaporated, a few drops of distilled water and a small quantity of *freshly prepared* zinc oxide added, gently boiled (water being added as necessary) for ten minutes, the three or four drops of liquid remaining filtered on to a glass slide, and allowed to crystallize. I obtained the forms seen in figure two.

Fig. 2

Their close resemblance to the crystals of the lactate of zinc (Fig. 1) will be seen at once. There can, in fact, scarcely be a doubt that they are lactate of zinc crystals. The lactic acid concerned in their formation must of course have existed in the carious dentine.

I have noticed in the dental journals a tendency on the part of some writers on this subject, to derive a large amount of satisfaction from the statement that, after all, what I have done to clear up the subject of dental caries was done and known long ago.

One writer even states that he might almost have said two years ago, something that I said but a few months since. Let me say, once for all, that I have too little spare time to devote any of it to the discussion of the question who *said* this or that first, or even who *might almost* have *said* something two years ago. There is perhaps no human disease about which more has been *said* than about caries of the teeth, and when the subject shall have received its final settlement there will be hundreds who may say "I told you so." Malassez and Vignal very justly say of Baumgarten,

who claims priority over Koch in the discovery of the tubercle bacillus: "*Il ne suffit pas de* trouver, *il faut* prouver"—and I do not hesitate to say with reference to some of the discussions which for years have been carried on concerning the cause of dental caries: *Il ne suffit pas de deviner, il faut trouver* et *prouver.*

It is not enough to guess the cause, or guess at it; we must *find* the cause, and, having found it, *prove* that it is the cause sought for.

If we infect a beef-extract-sugar solution with carious dentine, as described in the February number of this journal, using every possible precaution to obtain perfectly pure material and to prevent the access of germs from without, and keep the solution at thirty-seven degrees Centigrade, we may observe the following phenomena: In from eight to ten hours the solution will show a slight cloudiness, which at no time, however, amounts to complete opacity. Tested with sensitive litmus paper, it will be seen that the acid reaction has already appeared. In fifteen to twenty hours the fermentation will generally have reached the most active state, and soon afterwards a colorless, flocky precipitate will begin to form on the bottom of the vessel, accompanied by a corresponding clarifying of the solution, and a diminution of the fermentive activity. After the lapse of forty-eight hours the sediment will have completely formed, and the solution will be almost as transparent as when the experiment began.

The time required for the completion of this series of phenomena will, however, naturally depend somewhat upon the amount of dentine taken for the infection, and the amount of the solution used.

Impurities in the culture manifest themselves in various ways; it may be by an excessive cloudiness of the liquid, or by the formation of a skin upon the surface of the solution, or the failure of the latter to become clear after the regular lapse of time, etc., etc.

Dentine is an excellent medium for separating the different fungi found in the mouth, the most of them not being able to exist in the deeper parts, partly on account of the acidity of the medium, partly on account of the lack of free oxygen. We may, therefore, with the proper amount of care, obtain material of such purity as to produce a pure culture in the first generation.

If we microscopically examine the sediment which has formed on

the bottom of the vessel, we shall find it to consist of cocci and diplococci, either single or in chains; in either case without motion. Under a low power they appear round and regular; with $\frac{1}{18}$ oil immersion they are seen to be round or oval, regular or irregular, involuted, etc., presenting the most various shapes and sizes. I have never been able to detect the existence of spores, and reproduction takes place only after the scheme presented in figure one, Nos. 1, 2, 3, 4, 5, 6, 7. A coccus which may be round in the beginning, by extension in one axis becomes oval or elongated; soon after it shows a contraction in the middle, resulting in the production of a diplococcus, or two cocci, each of which may produce two cocci in the same manner.

Fig. 1

We find, consequently, in a chain taken from a growing culture, some of the cocci round, others oval, some of the diplococci but slightly contracted, while in others the contraction amounts almost to a complete division. (See Fig. 1, *d*, *e*, *f*.) Frequently the cells acquire a pronounced bacterium form, so that if they did not occur in the same chain with the ordinary forms, one would be in doubt as to whether they belonged to the same species.

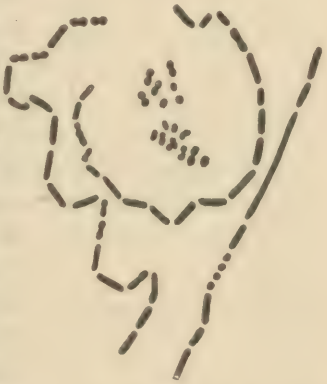
The growing cells in a chain sometimes turn upon their shorter axis, and then, growing out in the new direction, produce very peculiar figures. (Fig. 1, *f*, *g*.) In stagnant cultures the cells under high power are mostly very irregular, having in groups the appearance of the bones of the wrist. (See Fig. 1, *a*, *b*.)

Very characteristic are the involution forms produced both in stagnant cultures and in media which are not well adapted to the needs of the fungus. Here the forms and sizes are so various that it sometimes becomes exceedingly difficult, if not impossible, to tell if cer-

tain ones are normal or abnormal. (See Fig. 1, *h*, *i*, *j*, *k*.) In exceptional cases the threads surround themselves with a thick gelatinous sheath. (See Fig. 1, *c*.) The protoplasm of the involuted cells generally presents a granular appearance. (Fig. 1, *h*, *k*.)

If we make a large number of cultures at once we will, in about one case out of five to ten (and if the cultures are made in a decoction of malt, much more frequently), meet with a second fungus, essentially different from the one just described. It occurs chiefly in form of bacilli, but also as leptothrix, bacteria, diplococci and cocci singly, or, as is mostly the case, in long zigzag threads. (Fig. 2.)

Fig. 2



The discovery of this fungus, with its different forms of development, affords a very ready explanation of the fact that in a single dentinal tubule we sometimes find a transition from leptothrix to bacilli, from bacilli to bacteria, and from bacteria to cocci, an occurrence which I demonstrated nearly two years ago before the American Dental Society of Europe, before the Gesellschaft fuer Heilkunde, in Berlin, and to various private persons, including some of the most celebrated mycologists in Germany.

Those who maintain, as was done in the British Dental Association, that such cases may not be found, are responsible for their own mistake.

Macro-scopically, cultures of this fungus in beef-extract-sugar solution are not easily to be distinguished from cultures of that described above. The fungus collects as a sediment on the bottom of the vessel; it never forms a skin on the surface of the liquid, and produces but a moderate cloudiness of the same. In most decoctions, however, they present some peculiarities. Sometimes the fungus floats about in the solution in semi-transparent balls, or rises up from the bottom of the vessel like a miniature cloud of smoke, or collects in small patches on the sides of the vessel, while the solution itself remains almost perfectly clear. The cells are motionless, and do not form spores.

Fig. 3

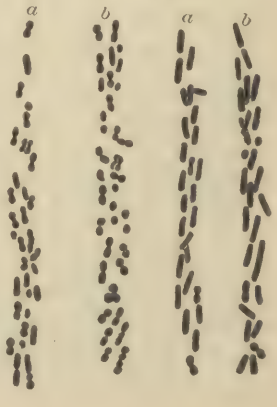
In order to discriminate between these two fungi I will designate for the present the one first described by the prefix *A* (alpha), and the one under consideration by the prefix *β* (beta). In all probability the *β* fungus also produces lactic acid from sugar. I say in all probability, because, though I have always been able to detect lactic acid in cultures of this fungus, I could not say with absolute certainty that cocci and diplococci of the species *A* were not present.

We have, then, in carious dentine two distinct fungi—one always, the other often, present; the former surely, the latter probably, producing lactic acid from sugar. If these fungi are the direct cause of dental caries, we should be able to produce caries by subjecting sound dentine to their action. This I have accomplished, as described in the March number of this journal.

In figure three *a* are seen in outline two tubules of dentine melted together by natural caries, and in figure three *b* two tubules melted together by artificial caries.

In figure four *a* are likewise two tubules from natural caries, and in figure four *b* two from artificial caries. It is a fact of considerable interest that, though the fungi themselves are perfectly colorless, pieces of dentine subjected to their action become yellowish, light brown, or dark brown, etc., depending upon the medium in which the culture is made, while different pieces of dentine in the same culture do not by any means necessarily acquire the same color.

Fig. 4



The carrying out of this experiment is attended with difficulties, and some may try it and fail. I have failed many times. The necessity of repeatedly changing the solution very much increases the danger from impurities; especially must the saccharomycetes be guarded against. The acidity of the medium caused by the caries fungi renders it very favorable for their development, and when they have once found their way into a culture it might as well be thrown away at once. Again, notwithstanding the presence of the pieces of dentine, the solution sometimes becomes sufficiently acid to impair, if not to destroy, the vitality of the fungus. In this case the dentine becomes softened, but only a slight invasion of the tubules takes place. Then, of course, in the very last stage of caries other fungi, especially leptothrix buccalis, are present in the decomposing dentine, and sometimes produce an appearance in its *superficial* layers, which I have not attempted to reproduce artificially. It is not difficult, by a simple microscopic examination of the fluids of the mouth, as well as of carious dentine, to find forms morphologically identical with those described above.

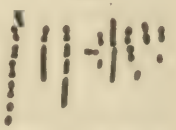
In figure five is seen in outline a portion of an epithelial scale from the human mouth, highly magnified, with the

Fig. 5

fungi lying upon the surface. The forms seen in figure six were obtained from a glass tube filled with starch and kept in the mouth over night, while figure seven is from carious dentine. The *A* caries fungus agrees morphologically with the fungus of sour milk as delineated by Pasteur. Later experiments, however, render it probable that the souring of milk is produced by an altogether different fungus, a short, thick *bacterium*, occurring in twos, seldom fours, which may also be found in the human mouth (though probably not deep in carious dentine), and will be considered at another time.

Fig. 6

Fig. 7



In the case of both fungi the *fermentation* goes on independently of the presence of free oxygen. I have already shown that where only a trace of oxygen is present, in no way comparable with the amount of acid produced, the degree of acidity was as great as where there was free access of air. Whether, however, this *trace* of oxygen is essential to the life of these fungi—*i. e.*, whether without it they would perish from asphyxia—is a question which we will not discuss here.

It has been generally supposed that the production of lactic acid by fermentation from sugar is accompanied by the evolution of carbonic acid; in fact, Fluegge says that no fermentation can go on without the production of carbonic acid. This statement will hardly be borne out by a study of the fermentation produced by the fungi of tooth caries.

A glass vessel of five hundred c. c. capacity was filled with beef-extract-sugar solution, infected with a pure culture of caries fungi and made air-tight with a rubber stopper, carrying an efflux-tube for collecting the gas over mercury. After twenty-four hours, during which time 1,75 c. c. acid had been produced, one single gas-bubble was collected, which may have been due to a slight change of temperature, as well as to a veritable gas evolution. The splitting appears therefore to be perfectly smooth, and to take place in accordance with the simple formula—

$$C_6 H_{12} O_6 = 2C_3 H_6 O_3.$$

It presents a marked contrast to the stormy character of the butyric and alcoholic fermentations, in case of which the pressure of the gas evolved is often sufficient to burst the vessels containing the cultures.

There is perhaps at nearly all times a sufficient amount of sugar in the oral cavity to enable the fungi of caries to carry out their characteristic ferment action. It remains, nevertheless, an interesting question, whether they have the power to form sugar out of starch; *i. e.*, whether they have any diastatic action. About thirty cultures in an aqueous solution of beef-extract and starch, and in a solution of starch in sterilized saliva, gave for the most part negative results; in exceptional cases a slight diastatic action appeared to take place, which I am inclined to regard as the result of some impurity in the culture, or an error in the experiment.

On the other hand, the fungi appear without doubt to possess the power to invert, or to render non-fermentable sugars fermentable, since cane sugar, which is not fermentable, and does not reduce alkaline solutions of sulphate of copper, acquires both these properties when subjected to their action. That this result is caused by the action of a ferment produced by the organisms, and which may be separated from them, is, I think, demonstrated by the following experiment: By making a number of cultures at one time in vessels of two hundred to five hundred c. c. capacity, and collecting the sediment which was deposited on the bottom of the vessels, I succeeded in bringing together a considerable quantity of the fungi; this was then treated with ninety per cent. alcohol, filtered and dried in a porcelain vessel, thoroughly rubbed with sand, digested with water at twenty-three degrees Centigrade, and again filtered; the filtrate (which must be clear, and should contain the ferment in solution) was added to a solution of cane sugar, which then showed, in the long tube of a Mitscherlich polariscope, a rotation equal to 5°,19. The solution was now allowed to stand four hours at a temperature of thirty-eight degrees Centigrade, after which time it produced a rotation of only 4°,54, indicating a decrease of about two-thirds of a degree. The solution also produced a slight reduction of an alkaline solution of sulphate of copper; *i. e.*, a certain portion of the cane sugar had been converted into invert sugar.

In the presence of the fungi the non-fermentable sugar, by the action of the invertine produced by the fungi, takes up one molecule of water and is coverted into invert sugar, a mixture of levulose and dextrose, both of which are fermentable.

$$C_{12}\,H_{22}\,O_{11} + H_2\,O = C_6\,H_{12}\,O_6 + C_6\,H_{12}\,O_6.$$

Cane sugar, Levulose, Dextrose.

We may say, therefore, that the micro-organisms require sugar to produce fermentation, but that it is immaterial which kind of sugar is furnished them. The fermentation is most active between the temperatures thirty-five and forty degrees Centigrade. Above fifty degrees and below fifteen degrees Centigrade, little or no production of acid takes place.

In addition to these two species of fungi, others of minor importance are occasionally met with in the mouth, and will receive attention at another time.

I would not have any one think that I look upon the above as a thorough consideration of the fungi of tooth caries. To me it appears very imperfect. Nevertheless, I thought it well to present the matter before the profession in the hope that others might be induced to take it up and help to complete the work thus begun. In the next number I will present the results of experiments relating to the action of various antiseptics, filling materials, etc., upon the fungi under consideration.

THE INFLUENCE OF ANTISEPTICS, FILLING MATERIALS, ETC., UPON THE FUNGI OF DENTAL CARIES.

Having established upon an experimental and scientific basis the fact that caries of the teeth is, to a certain extent, the direct result of the action of ferment acid or acids* upon the tissue of the tooth, followed, particularly in the case of the dentine, by the action of the ferment organisms themselves upon the decalcified tissue, it becomes a matter of the first importance to determine, first, by what means we may counteract the action of the acids or prevent their production; second, by what means we may save the already decalcified dentine from complete destruction.

* The chief work in the production of caries is performed by lactic acid; other acids are only auxiliary factors.

Evidently there are three methods by which the desired end may be partially obtained:

1. By repeated, thorough, systematic cleansing of the oral cavity and the teeth, we may so far reduce the amount of fermentable substances in the mouth and the number of ferment organisms, as to materially diminish the production of acid. This is so self-evident that it needs no further comment.

2. By the repeated application of alkaline substances we may, to a certain extent, neutralize the acids before they have acted upon the teeth to any considerable degree.

3. By a proper and intelligent use of antiseptics we may destroy the organisms themselves, or at least render them inactive. It is this method which is especially applicable in the second stage of dental caries (*i. e.*, the stage which follows the decalcification), and to which we will here give exclusive attention. We must, however, constantly bear in mind that by whatever method we proceed, a previous thorough cleansing of the teeth is absolutely indispensable. There is no known solution, alkaline or antiseptic, applicable in the human mouth, which will penetrate between the teeth or to the bottom of fissures and cavities, when these are filled with food, in sufficient quantity to have any appreciable effect. Therefore, before all antiseptics or alkaline washes come the toothbrush, toothpick, and floss silk.

In my experiments for determining the action of various antiseptics upon the fungi of tooth caries, it appeared to me that by allowing the antiseptic to act upon the fungi in their natural medium, saliva, I could obtain results of more practical value than by experimenting upon them in artificial solutions, and in pure cultures, neither of which ever occurs in the human mouth. Furthermore, since the fungi can attack the teeth only after a partial decalcification, we have in the first place to demand of an antiseptic, not so much that it destroys the fungi, as that it prevents the production of acid by them.* Consequently, if an acid reaction failed to appear in a solution of saliva and sugar to which a certain anti-

* The production of acid may be taken as synonymous with the development of the fungi, though the failure of the acid reaction to appear after a certain length of time does not necessarily indicate that the fungi have been *devitalized.*

septic had been added, as soon as in a like solution to which no antiseptic had been added (control), it was taken as evidence of the activity and value of the antiseptic used. This method could of course be used only with substances having a neutral reaction. The solutions were also subjected to a microscopic examination, to render the evidence doubly sure.

In the following table I have indicated the percentage of each antiseptic experimented upon which must be present in a sweetened-saliva solution, to prevent the appearance of an acid reaction in twenty-four hours, or in case of alkaline or acid antiseptics, to prevent the development of the characteristic fungi in the same time.

For example, if to 100,000 parts of sweetened saliva we add one part of bichloride of mercury, the solution will not be found acid after the lapse of twenty-four hours, even though the control became sour in four or five hours. If we add only one part to 500,000, the acid reaction will appear somewhat later than in the control.

This table is designed to show the comparative strength of the antiseptics most commonly used. The action of the antiseptics having an acid or alkaline reaction upon the fungi, was determined by the use of the microscope alone.

	Production of Acid (Development of Fungi)	
	Prevented.	Retarded.
Bichloride of mercury	1–100,000	1–500,000
Nitrate of silver	1–50,000	1–100,000
Iodoform	1–5,000	1–10,000
Naphthaline	1–4,000 (?)	1–9,000
Iodine	1–6,000	1–15,000
Oil of mustard	1–2,000	1–5,000
Permanganate of potas	1–1,000	1–2,000
Eucalyptus oil	1–600	
Carbolic acid	1–500	1–1,000
Hydrochloric acid	1–500	1–1,000
Phenylic acid	1–200	1–500
Liquid of Agate Cement	1–250	
Liquid of Excelsior Cement	1–225	
Lactic acid	1–125	1–250
Carbonate of sodium	1–100	1–200
Salicylic acid (Conc. alcohol sol.)	1–75	1–125
Alcohol	1–10	1–20

The experiments show that bichloride of mercury is about two hundred times as powerful as carbolic acid, and demonstrate very clearly the mistake of substituting weak solutions of this antiseptic (1–1,000, as I have seen recommended) for concentrated carbolic acid. One one-thousandth is only one-fifth as powerful as pure carbolic acid, which in many cases may be used with impunity. It is consequently useless to attempt to introduce the sublimate solution for the purpose of sterilizing root canals, cavities before filling, etc., unless we may use at least a one-half per cent., if not a one per cent. solution. I see no reason, however, why this may not be done. In a few cases I have used a one per cent. solution for treating root canals, and do not hesitate, particularly with the rubber dam adjusted, to wipe out cavities before filling with a two per cent. solution, and see no possible evil which could result from it. A well-known physiologist in Berlin has told me that he uses a one per cent. solution in his own mouth for aphthæ, and with excellent results. We should not, however, overlook the fact that a one per cent. sublimate solution is only one-fifth as powerful as pure iodoform.

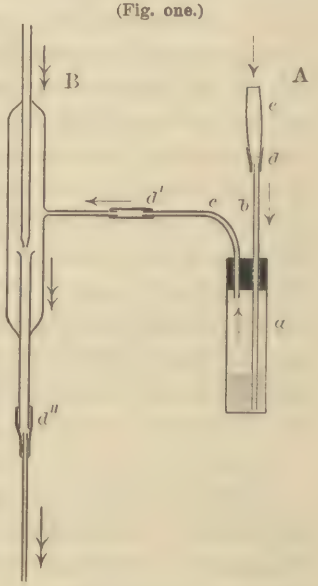
As a mouth wash I have frequently used a one-tenth per cent. (1–1,000) solution myself, and have seen no bad results from it; I would not, however, recommend it to my patients in this strength. It has, besides, for me, an exceedingly disagreeable and lasting taste, which it is difficult to disguise, and produces an immediate increased secretion of saliva and mucus, which is very annoying. A one-fiftieth per cent. solution (1–5,000) may eventually be brought into use; in this concentration it is four times as powerful as a one per cent. solution of carbolic acid. The very high antiseptic power of nitrate of silver is particularly noteworthy. Why may it not be employed in place of the much more dangerous mercuric chloride?

The action of tobacco upon the fungi is worthy of notice. Five grammes of old Virginia plug were boiled fifteen minutes in fifty c. c. of water, the loss by evaporation being constantly replaced; the decoction was then filtered, and a portion added to an equal volume of saliva with sugar. This produced a mixture scarcely stronger than that which many veteran chewers carry around in their mouths all day, and in it the fungi led only a miserable existence.

Much more remarkable, however, was the action of tobacco smoke upon the fungi; the smoke from the first third or last quarter of a Colorado Claro cigar being found amply sufficient to sterilize ten c. c. of a beef-extract-sugar solution, previously richly infected with caries fungi.

The apparatus used for this experiment (see figure one) explains itself. A current of water passing through the part B in the direction of the ↘ produces a current of air through the part A, in the direction of the ↘ which draws the smoke from a lighted cigar through the solution. The rate at which the cigar smokes may be regulated at will by the cock of the hydrant.

(Fig. one.)



a, Glass cylinder with infected solution.
b, *c*, Glass tubes.
d, *d'*, *d''*, Rubber tubing.
e, Cigar (Colorado Claro.)
B, Water air-pump.
A current of water passing through B in the direction of the ↘ produces a partial vacuum in the bulb, and consequently a current of air in the direction of the ↘ or through the cigar, which if lighted will smoke at a rate determined by the pressure under which the water is flowing.

In consideration of the strong antiseptic power of tobacco smoke, we might be inclined to infer that tobacco smokers should never suffer from caries of the teeth; it is evident, however, that there are very many points in the dental arch to which the smoke never penetrates.

In the preparation of cavities for inserting fillings, it is naturally often next to impossible to remove all the carious dentine, and in all such cases it is especially desirable that the filling material itself should possess antiseptic properties, since we, in using such a material, not only destroy those organisms existing in the carious tissue, but the material, if it remains permanently antiseptic, retards the working of the ferment organisms from without, and the appearance of secondary decay. We need, therefore, a material for filling which is not only antiseptic at the time of insertion, but which remains permanently so after being inserted.

I have endeavored to determine the relative antiseptic power of different filling materials (cements, amalgams, etc.), not only at the moment of mixing, but after they were thoroughly dry, after they had lain some hours in sweetened saliva, and after they had been an indefinite time in the human mouth.

A large number of miniature test-tubes (homeopathic pill-tubes) were provided with cotton stoppers, and sterilized. Into each was brought one-half c. c. of beef-extract-sugar solution, previously infected with carious fungi (pure culture). To the first tube was added a small drop of a one per cent. sublimate solution; the second tube was left untouched, and into the third, fourth, fifth, etc., were brought the filling materials whose antiseptic virtues were to be tested; these were in the form of cylinders two m. m. in diameter, and three m. m. long; if old fillings from the mouth were used, pieces were taken having approximately the same size.

These tubes now being placed in the incubator, their contents became cloudy one after the other. In those tubes which contained fillings of but slight antiseptic power, the development of the fungi proceeded rapidly, and the cloudiness soon appeared. If, on the other hand, the filling was strongly antiseptic, the development of the fungi was hindered, and the cloudiness appeared later. The first tube to which the sublimate solution had been added of course remained clear, and by comparing the others with this it was easy to see just when the turbidity began to show itself; the second tube, containing no antiseptic and no filling, served as control, and the space that intervened after the control became turbid till any one of the other tubes became turbid, was a measure of the antiseptic power of the material in that tube.

As the result of a great number of experiments, I have been able to get together the following table:

When the control tube becomes turbid in 5 hours, then:

A tube containing an old oxy-phosphate filling becomes turbid in	...	5	hours.
" " " oxy-chloride " " " "	...	5	"
" " a gold cylinder becomes turbid in	.....	5	"
" " a Hill's stopping cylinder becomes turbid in	.....	5	"
" " an amalgam cylinder (kept 12 hours in saliva) becomes turbid in	.....	5½	"

A tube containing	an agate cylinder (kept 12 hours in saliva) becomes turbid in	$5\frac{1}{4}$	hours.
" "	an old amalgam filling becomes turbid in	$5\frac{5}{10}$	"
" "	an amalgan cylinder (mixed dry) becomes turbid in	$5\frac{8}{8}$	"
" "	an amalgam cylinder (mixed wet) becomes turbid in	$5\frac{1}{2}$	"
" "	an oxy-phosphate cylinder (12 hours in saliva) becomes turbid in	$5\frac{1}{2}$	"
" "	an amalgam cylinder (12 hours old) becomes turbid in	$5\frac{3}{4}$	"
" "	an old filling of tin and gold becomes turbid in	$5\frac{1}{4}$	"
" "	an oxy-phosphate cylinder (12 hours old) becomes turbid in	6	"
" "	an agate cylinder (12 hours old) becomes turbid in	$6\frac{1}{4}$	"
" "	an iodoform cement cylinder (12 hours in saliva) becomes turbid in	$6\frac{3}{4}$	"
" "	a pyrophosphate cylinder (mixed dry) becomes turbid in	$7\frac{1}{8}$	"
" "	a pyrophosphate cylinder (mixed wet) becomes turbid in	$7\frac{3}{8}$	"
" "	an oxy-chloride cylinder (12 hours old) becomes turbid in	9	"
" "	a piece of dentine from a tooth impregnated by a copper amalgam filling becomes turbid in	11	"
" "	an iodoform cement cylinder (12 hours old) becomes turbid in	12	"
" "	an iodoform cement cylinder (fresh) becomes turbid in	?	"
" "	a globule of mercury becomes turbid in	—	"
" "	a cylinder of black oxide of mercury becomes turbid in	—	"
" "	a cylinder of any copper amalgam becomes turbid in	—	"
" "	any old copper amalgam filling becomes turbid in	—	"
" "	a cylinder of oxy-chloride (fresh) becomes turbid in	—	"

The (—) signifies that the solution remained permanently clear.

We see from these results that the only filling at present in use which exerts a continual anti-ferment* action upon the walls of the

* I use the terms anti-ferment and anti-septic interchangeably, though the former is, perhaps, preferable, since we are treating of ferment, and not septic organisms.

tooth and its immediate surroundings, is the old copper amalgam; not only that, but the very substance of the tooth containing such a filling itself becomes antiseptic, a piece of bluish or bluish-green dentine from such a tooth very powerfully retarding the development of the fungi, and, indeed, in two cases completely destroying them. Secondary decay in such a case would be next to impossible, where anything like cleanliness was observed.

This result is well supported by observations which I have had abundant opportunity to make for the last five years, here where this material is so extensively used, and I do not hesitate to say that if our only object is to check the destruction of tissue by caries, there is no material at present in use with which this object may be so surely accomplished as with a good copper amalgam. It is a material, however, which I have never used, though I am not aware of any bad effect produced by it beyond the discoloration of the tooth. Skogsberg's iodoform cement came into my hands too late to complete the experiments with it. It has undoubtedly strong antiseptic properties, which it does not completely lose even when exposed to the saliva, and might no doubt be used to great advantage as a foundation for permanent fillings. Old fillings of tin and gold possess slight antiseptic power, still less (almost zero) old amalgam fillings (not copper). The very inconsiderable power of amalgams to prevent the development of ferment fungi is a source of some surprise, since we have been accustomed to look upon them as very active in this respect. It is probably a mistake to attribute the hardening of dentine under amalgam fillings to the antiseptic action of the amalgam, since, in the first place, it possesses this power to but a slight degree, and in the second place the hardening may take place under fillings of gutta percha equally well. If we dry the cavity but indifferently well, and then choose a piece of gutta percha which we think will about fit the cavity, warm it and stuff it into the cavity, we of course can expect only bad results. If we proceed as follows we will obtain excellent results, as I have seen time and again: Adjust the dam, excavate carefully, especially the margins, wash with a strong antiseptic, dry thoroughly with bibulous paper and then with the hot-air syringe, till the sur-

face of the dentine becomes whitish, paint with a thin solution of copal varnish, dry again with warm air, then put in the gutta percha in small pieces, one after the other, being *sure* that each piece sticks to its place, especially along the margin, just as if you were making a filling of gold. A piece which has once moved in its place must not be allowed to remain, as a leak will be the result. Remove such a filling after two years, and the cavity will often be found in an excellent condition for a gold filling.

The oxy-chlorides when first mixed, are powerfully antiseptic, but soon lose their energy when exposed to the action of saliva.

The oxy-phosphates are very much inferior to the oxy-chlorides in antiseptic power, and should never be used in cavities where there is much soft dentine. This conclusion is borne out by my own experience in practice, and by that of others with whom I have conversed on the subject. Dr. Paetsch first called my attention to the disastrous results of such a practice, and his testimony was confirmed by that of Dr. F. P. Abbott, and others.

It must not be expected that the results given in the above table are absolutely free from error. The experiment is attended with more difficulties than are at first sight apparent; especially does the sterilization of the filling materials themselves involve much time and labor, and the results are not always constant; this was especially the case with iodoform cement. Amalgams and phosphates gave quite constant results. The tests with some of the materials were made over twenty-five times; with others, such as copper amalgams, where there was no doubt as to the result, only a few experiments were made.

Caries of the teeth, except in the later or last stage, is the result of a ferment process, and the organisms found in the deeper parts of decaying dentine, which I have isolated and obtained in pure culture, are ferment organisms. The decomposition of the pulp and contents of the root canal, attended by bad-smelling products, is, on the other hand, a putrefactive process, in which entirely different species of fungi are concerned. Whether or not the results which I have obtained for the fungi of caries would apply equally

well to those putrefactive fungi, is a question which can be settled only by experiment upon pure cultures of the same.

Although I have now, as I think will be granted, established upon a sure basis a fact that caries of the teeth may result directly from the action of acid-producing fungi in the presence of fermentable carbo-hydrates, the conclusion would hardly be justifiable that, by keeping the mouth constantly and perfectly free from all fermentable substances, or by repeated application of antacids or antiseptics to all parts of the teeth, or by all these means together, we could ever banish dental caries from the oral cavity. A most powerful influence, which we do not well understand, is exerted by the nutritive processes in the teeth themselves.

I am assured by men who have grown old in the practice of dentistry, that mouths which have long been under their observation, and which practically have been completely free from caries for years, at once, on account of some sudden change of health, show a general breaking down or crumbling of the teeth, *en masse*, in the space of a few weeks. It has also been my experience that patients who have been dismissed by their dentists in America, with the assurance that according to previous experience their dentures would require no treatment for one or two years, have come to me a few weeks later with teeth looking as though they had not been under the hands of a dentist for years. Some say the ocean voyage spoiled their teeth; others attribute it to a change in the climate, food, health, etc.

At any rate, we have here a cause which lies without the domain of both bacteria and acids (either ferment or otherwise). The lime-salts of the teeth are supposed to form, with the organic matter of the tooth, a definite chemical compound, and it is probably due to this fact that simple salts of lime are so much more readily soluble in weak acids than pulverized tooth-bone, or that the tartar upon the teeth is so much more easily soluble than the teeth themselves; so that when any one rinses his mouth with vinegar, and afterwards finds lime in the vinegar, we know that the lime, in by far the greater part, if, indeed, we may not say altogether, came from the tartar. Now, though there is no positive evidence for the supposi-

tion, it is certainly not altogether improbable that, as a consequence of certain derangements in the nutritive functions of the teeth resulting from a change of health, etc., etc., a dissolution of the affinity between the lime-salts and the organic matter may take place, thus setting free the easily soluble lime-salts, which are then carried away in solution or washed out mechanically.

This is a supposition only, which I bring forward because facts in this case are absolutely wanting. If it should, perchance, contain a trace of truth, then adult and pulpless teeth should be less subject to these *sudden* attacks of caries than young teeth with living pulps.

There still remains much hard work to be done, before the subject of dental caries may be dismissed as having received a final solution *in all its different phases.* There are men enough in the profession, however, who are willing to work, and who do not shrink from the tasks yet to be performed.

THE FUNGI OF DENTAL CARIES; THEIR PURE CULTIVATION AND EFFECT UPON LOWER ANIMALS.

In the May number of the INDEPENDENT PRACTITIONER will be found the description and illustrations of two species of micro-organisms which I had, up to the first of March, 1884, obtained from carious dentine. These species I isolated by inoculating culture liquids with very small pieces of carious dentine, taken from near the border of the normal tissue. If the fungus was not at once obtained in the pure state, a second culture tube was inoculated after the method of fractional culture, with a minimum portion of the first, and so on.

It soon, however, became apparent that the capture of these two species by no means ended the work; on the other hand, new forms continually presented themselves, and in order to be able to determine definite characteristics for each species, resort was had to the culture on plates of gelatine prepared with beef extract, calf's broth, malt decoction, etc.

The beef-extract gelatine, for example, I prepare as follows: 200 c. c. water + 3,0 beef extract + 3,0 sugar, are first neutralized,

then slowly boiled for five minutes, and filtered (filter and all other vessels, of course, sterilized) After cooling, 8,0 of the finest gelatine is added and gradually heated till the gelatine is dissolved ; it is then cleared with the white of an egg, and all together kept at the boiling point for about five minutes, stirring constantly to prevent burning ; it is then passed through a filter, surrounded by a bath of boiling water, into glass tubes with cotton stoppers (both sterilized), and kept in a refrigerator. When to be used it is melted in warm water and poured upon sterilized cold glass plates, which may be m. 0,15 long by m. 0,07 wide, and placed in the moist chamber. The layer of gelatine should be about two m. m. thick.

Suppose now we have a culture containing different species of fungi, and we wish to separate them. A thin platinum wire with one end melted into a glass rod is sterilized in the flame of a bunsen burner, and on cooling dipped into the impure culture and lightly drawn across the surface of the gelatine; the fungi which adhered to the platinum wire are thereby scattered in a row upon the surface of the gelatine, and in a short time we will find that at certain points in the row one form of fungus has developed, and at other points other forms. Now if we take upon the end of our platinum wire a small quantity of fungi from one of these points and draw it across the surface of a second plate, we will, in parts of this line, invariably obtain a pure culture of one of the species in the original impure culture, nearly every species being distinguished by some characteristic in the form which it takes in growing, and in its action upon the gelatine. Having obtained a pure culture in this manner, test-tubes containing gelatine are inoculated with it. In these it may be kept in a pure state for weeks, or months, while the plates are always short-lived.

The gelatine method of pure culture has one great disadvantage in the low melting point of the gelatine. Twenty four to twenty-five degrees Centigrade is the highest temperature to which they can be exposed without danger of melting, and this, to fungi which are accustomed to a temperature of thirty-seven degrees Centigrade, is not always a matter of indifference. I have succeeded in isolating three species, besides

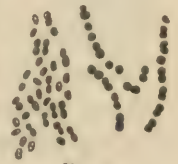


Fig. 1

the ones described in the May number of this journal, and, for the purpose of distinguishing them only, I will designate them by the Greek letters γ, δ and ε. These fungi are shown in Figs. 2, 3 and 4. In Fig 1 I have reproduced the fungus described on page 226 as a caries fungus, for the sake of comparison. When the species α, γ and δ are isolated, it is not difficult to tell one from the other; when, however, they are mixed together, it is next to impossible to determine which is which, and especially is this the case with α and γ. Their modes of development on gelatine are, however, so different, that we possess therein a ready means of distinguishing between them.

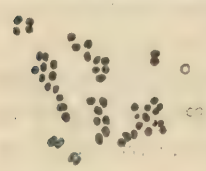


Fig. 2

The α fungus, *sparingly* inoculated into gelatine tubes, presents in a few days the appearance which I have attempted to represent in Fig. 1α. It may be compared to a bunch of grapes, which presents all gradations from the fully developed berry to the little green one; the masses of fungi are globular or ovoid, exceedingly fine, and semi-transparent, presenting altogether a strikingly beautiful culture, which it is impossible to even approximately represent by drawing.

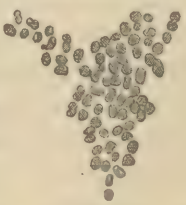


Fig. 3

Fig. 1α

Fig. 2α

Fig. 3α

It furthermore forms a button upon the surface of the gelatine; the latter becomes softened but not liquefied. On the plates it presents soft, milky ridges or knots, raised sometimes a m. m. above the surface of the gelatine, and obtaining a width at the base of three to six m. m. The γ fungus differs from all other fungi that I have yet found in decaying dentine, in that it completely liquefies the gelatine. The culture tubes present, therefore, a funnel-shaped area of liquefied

gelatine, while the fungi themselves fall to the bottom of the funnel (see Fig. 2*a*).

This fungus forms furrows in the plates, and if the plate is turned on its edge the whole mass of fungus flows from one end of the furrow towards the other, or slides quite off the plate.

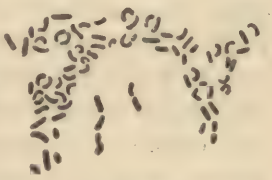


Fig. 4.

The *b* fungus (Fig. 3) forms completely *opaque* masses which may have a slight yellowish tinge, provided the gelatine itself is yellowish. It has a small surface growth, and liquefies the gelatine only to a slight extent. In cultures on plates which are two or three days old, the row of fungus appears to lie in a trough, or depression in the gelatine. It does not move, however, when the plate is turned on edge (see Fig. 3*a*).

For the fungus of Fig. 4 I have not yet been able to establish definite peculiarities of growth. As far as my observations have at present extended, it differs from that of Fig. 3, in that it is almost entirely wanting in surface growth, and forms colorless masses, even in colored media. It does not liquefy the gelatine. Viewed by transmitted light it appears to have a bluish tinge, and a slight opalescence. It grows, however, very slowly, and I have consequently as yet been unable to establish certain and definite characteristics for it. The fungus described on page 227 grows still more slowly at gelatine temperature, and I cannot at present give any microscopical features by which cultures on gelatine may be distinguished.

The most important feature connected with all these fungi, especially the coccus forms, is that they possess a ferment activity ; in other words, they are capable of producing acid out of sugar, or, in the human mouth, out of starch, by the aid of the diastatic action of the saliva. They may consequently all be looked upon as factors in the decay of the teeth. I would not venture to say that the *a* fungus is more concerned in the process of caries than all the rest together; nevertheless, such is the constancy with which I have found it, that if any one else should make the assertion I would

have no reason for contradicting him. Cultivated in liquid substrata, none of them form films or skins upon the surface of the liquid, but powdery or fleecy precipitates upon the bottom and sides of the vessel. None, so far as I have observed, produce an evolution of carbonic acid in solutions containing sugar, nor do they appear to suffer when the access of oxygen is restricted.

A question of great importance, not only for dentists but for general physicians, and, in fact, for everybody, is that relating to the possible pathogenic nature of these fungi. We find in the works of Leyden and Jaffé, Haussmann, Bollinger, James Israel, etc., sufficient ground for the statement that "these fungi, in all parts of the human body which they reach, can play the same malignant role as upon the teeth." Gangrene of the lungs, abscesses of the mouth and throat, chronic pyæmia, etc., etc., have by various authors been ascribed to the action of the fungi of the human mouth. Raynaud, Lannelongue, and Pasteur produced what they called *maladie nouvelle* by inoculating rabbits with the saliva of a child bitten by a mad dog. And A. Fraenkel has in a number of cases produced sputum-septicæmia by inoculating rabbits with his own saliva.

We ask ourselves then the question: may not many of our obscure cases of infectious disease which now and then appear after extraction, or other dental operations, and which are, without further examination, attributed to the unclean instruments or hands of the dentist, be the result of an infection produced by micro-organisms in the patient's own mouth? If a man's saliva contains organisms which, when brought into the blood of a rabbit, occasion death in twenty-four hours, would it be a matter of no consequence to produce so large a wound in his mouth as that caused by the extraction of a tooth? For the purpose, if possible, of throwing some light upon this question, I have undertaken a series of experiments for determining whether the organisms which are most commonly found in the human mouth possess the power of producing death (by septicæmia or otherwise) by inoculation. These experiments, as well as the others recorded in this article, I have in fact only begun. My absence from home, however, pre-

vents my carrying them on during the summer months, and I have determined, therefore, to present the results which I have already obtained, few and imperfect as they are.

The inoculations have thus far been performed on three rabbits, one rat, and six white mice. They were made partly with a mixture of the two fungi α and γ, and partly with saliva which had been kept in sterilized calf's broth for fifteen hours, at blood temperature.

Each rabbit received 1 c. c. of the infected liquid, injected directly into the lung or abdominal cavity; the rat 0,2 c. c., and the mice 0,1 c. c.

Exp. 1. Small rabbit inoculated with 1 c. c. in the abdominal cavity.

In the course of a few hours the rabbit appeared evidently ill; refused to eat, and remained quiet in the corner of the cage. In twenty-four hours diarrhœa appeared, with a slight elevation of temperature. These symptoms increased during the next day, till fifty hours after the time of inoculation it was found at the point of death. The examination showed the blood to be almost entirely free from organisms, and no indication of septicæmia. Living fungi were found, however, in the abdominal cavity, and a large part of the right lobe of the liver was completely riddled with masses of fungi; also in the fæces were found enormous numbers, which, morphologically, were identical with those in the liver, their entrance into the alimentary canal from the liver being easily accomplished. I unfortunately neglected, however, to establish their identity by the proper cultures.

Exp. 2. Rabbit inoculated as in Exp. 1.

The animal manifested a slight indisposition on the second day, from which it soon recovered.

Exp. 3. Rabbit inoculated in the right lung with saliva which had been kept in sterilized calf's broth for fifteen hours, at thirty-seven degrees Centigrade. No effect apparent.

Exp. 4. White rat, injection in abdominal cavity.

The animal remained well.

Exps. 5–11. Seven white mice; five inoculated in abdominal

cavity with α and γ fungi ; two in the lungs with saliva in calf's broth. Of the former two died at about the fortieth hour under the same symptoms as in Exp. 1. Great numbers of fungi were found in the abdominal cavity, which by culture on gelatine proved to be the γ fungus. A number of colonies were likewise found in the liver. Microtome sections of the liver of the rabbit stained in Fuchsine show, when examined under the microscope with sufficient light to drown the tissue, a distribution of the fungi very similar to that often seen in the outermost layers of carious dentine. (See Fig. 5.) Of course, no definite conclusion can be drawn from a few experiments. They are, however, sufficient to show that these fungi certainly do possess a pathogenic character, and when brought into other parts of the human body may be able, under predisposing conditions, to produce disastrous results. Especially the continual swallowing of these fungi in great numbers may, by their ferment activity alone in the course of time, produce very serious derangements of the stomach and alimentary canal, since the small percentage of hydrochloric acid in the stomach, even in the presence of the normal quantity of pepsin, is not sufficient to devitalize them. It was with a certain degree of satisfaction that I have failed thus far to find the coccus of sputum-septicæmia in my own saliva. It is, however, very desirable that experiments should be made with the saliva of many persons, for the purpose, if possible, of determining in what proportion of cases this fungus is present.

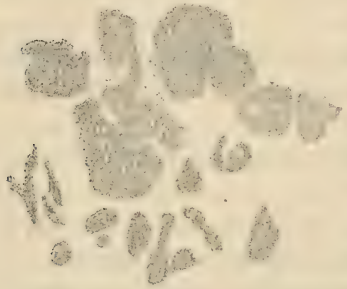


Fig. 5

Messrs. Underwood and Milles have endeavored to repeat some of my earliest experiments in the production of artificial caries, but under those very abnormal conditions against which I entered warning in the Independent Practitioner, page 229. Failure was the necessary result. They performed, further, a very elaborate experiment, lasting six months, in which the baths became so

putrid and offensive that "they quit the experiment with relief." They naturally produced no caries, thereby furnishing an admirable confirmation of the fact to which I have so often called attention, that it is impossible to produce even a trace of caries by putrefaction alone. They tried a third experiment, putting the fungi under such abnormal conditions that they could not produce acid, and of course failed again, once more confirming the fact that I have long since established, that we can have no caries without acid. With these experiments they risk the statement that artificial caries is probably an impossibility. The production of artificial caries is a *fait accompli*, and to deny its possibility is only to endanger the reputation of him who denies.

They state further that they can find no softened dentine which does not contain micro-organisms. This, however, is contrary to the experience of a great many American microscopists, and, moreover, as I have elsewhere stated, I shall take with me to the next meeting of the American Dental Society of Europe several hundreds of specimens of carious dentine, and be ready to show the areas of softened, non-infected dentine, on any one or on all of them.

Messrs. Underwood and Milles understand me, in the third place, as being of the opinion that all the micro-organisms connected with caries of the teeth are only different forms of one fungus. The readers of the INDEPENDENT PRACTITIONER know better. I have stated simply that *one* of the many fungi found in the human mouth in connection with caries of the teeth, may produce different forms of development. This is the fungus which I have designated by the prefix *β*. It is scarcely necessary to add that I am always prepared to prove its existence microscopically, as well as on the authority of many of the best mycologists of Germany.

No one, I think, will deny that within the last few years I have done a large amount of work, and contributed some evidence towards the solution of the problem of dental caries. The amount of material dealt with, and the ground gone over, have been so extensive that it has been absolutely impossible, with the greatest efforts, to remain as long by each step as would have been desirable. It

may be, therefore, that at some points the subject has not been presented with sufficient clearness or decisiveness; it may be, too, that at some points the conclusions have been faulty, since I make no pretension to infallibility. Time will show whether this is the case. At present I know of no important change which I could make, if I were to re-write all my contributions of the last three years.

I desire to give, in closing, a very short resumé of the work which I have accomplished.

1. I convinced myself by the examination of some thousands of slides of carious dentine, that micro-organisms were always present, and that they, without any doubt, were the cause of various anatomical changes which were found to take place in the structure of the dentine during caries. (Here, of course, the question of priority does not suggest itself; Leber and Rottenstein, as is well known, were the first to give definite expression to this fact.)

2. I proved, at the same time, that the invasion of the micro-organisms was not, in the majority of cases, simultaneous with the softening of the dentine, but that large areas of softened dentine could be found that contained no fungi. Of all those who examined my preparations in America, no one, whatever his theory, ever once denied this fact. I concluded from this that the softening of the dentine went in advance of the invasion of the organisms.

3. I determined by analyses of masses of carious dentine, sufficiently large to give reliable results, that the softening of the dentine is of the nature of a true decalcification. That the decalcification of the outer layers is almost complete, and diminishes in degree as we advance towards the normal dentine. Furthermore, that the same relations maintain in dentine softened in a mixture of saliva and bread, or in weak organic acids; also, that in a mass of carious dentine the lime-salts had been removed to a much greater extent than the organic matter.

4. I maintained from the first that the softening of the dentine was produced by acids, for the most part generated in the mouth by fermentation. I had, however, no direct proof of this.

5. I *proved* that fungi exist in great numbers in the human

saliva and in carious dentine, which have the power to produce acid under conditions which are constantly present in the human mouth. I determined this acid, for one of the fungi, at least, to be the ordinary ferment, lactic acid.

6. I produced caries artificially, which under the microscope cannot be distinguished from natural caries, by subjecting sound dentine to the action of these fungi in fermentable solutions.

7. I determined the influence of various antiseptics and filling materials upon the fungi of caries.

8. I isolated various forms of these fungi, and determined *in part* the conditions most favorable to their development, their characteristic reaction upon gelatine, their physiological action, their effect when inoculated into the system of lower animals, and their possible connection with certain obscure diseases generally attributed to the carelessness of the dentist.

My continual search has been after facts, and such facts as I have obtained I have presented before the profession, never putting before them either theory or speculation, nor anything which was not the result of severe and continued labor, and in this spirit I propose to prosecute this work, as well as any other that I may undertake in the interest of the profession.

BERLIN, May 21, 1884.

NOTE.—Since writing the above, I have succeeded in producing death by septicæmia of both mice and rabbits, by injecting into the lung saliva from the mouth of a perfectly healthy person.

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